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Epstein-Barr Virus Infection and Associated Products (LMP, EBNA2, vIL-10) in Nodal Non-Hodgkin's Lymphoma of Human Immunodeficiency Virus-Negative Japanese

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Sixty cases of B-cell nodal non-Hodgkin's malignant lymphoma (B-ML), and 46 cases of T-cell nodal lymphoma (T-ML) were surveyed for Epstein-Barr virus (EBV) genomes, RNA, and associated proteins. We used a Southern blot analysis, polymerase chain reaction (PCR), and EBV-encoded small RNA-1 (EBER-1) in situ hybridization to investigate the presence of EBV. We performed an immunohistochemical study on EBV-related oncoproteins, such as EBV-determined nuclear antigen-2 (EBNA-2), latent membrane protein (LMP), and viral interleukin-10 (vIL-10). In addition, we also analyzed the terminal repetitive sequence of EBV (EBV-TR) to investigate the EBV-infected cell clonality. Non-Hodgkin's lymphomas were grouped into three types by number of EBV-infected cells: I) almost all lymphoma cells showed an EBV presence; II) some scattered lymphoma cells showed an EBV presence; and III) only a few cells showed such a presence, which was probably due to a latent EBV infection. In 25 of 60 B-MLs, EBV-infected cells were found; 7 were type I, 1 was type II, and 17 were type III. In 27 of 46 T-MLs, EBV-infected cells were found; no cases were type I, 5 cases were type II, and 22 cases were type III. Seven B-MLs and 3 T cell lymphomas showed clonal TR bands. Expression of EBNA-2 was found in only three B-MLs, whereas LMP was seen in four B-MLs and six T-MLs. All EBNA-2/LMP-positive cases showed an EBV presence. In B-MLs, expression of EBNA-2 and LMP was detected in almost all lymphoma cells; in T-MLs, however, LMP was found in only a small portion of the lymphoma cells. Expression of IL-10 was closely associated with LMP. In summary, it was thus speculated that EBV infection was associated with the various states of lymphomagenesis. © 1996 Wiley-Liss, Inc.

Key words: non-Hodgkin's lymphoma, EBV, LMP, EBNA-2, vIL-10

INTRODUCTION

Epstein-Barr virus (EBV) is a herpes DNA virus that is well recognized for its oncogenic properties. Infectious mononucleosis, Burkitt's lymphoma, nasopharyngeal carcinoma, and secondary B-cell proliferation in immunosuppressed individuals have all been well demonstrated to be associated with EBV [1,2]. EBV genomes have been detected in Hodgkin's disease (HD), and the viral genomes were present as episomes of monoclonal origin confined to Hodgkin's and Reed-Sternberg cells, which constitute the malignant cell population of HD [3,4]. More recently, EBV genomes have also been found in the nuclei of adult T-cell leukemia/lymphoma (ATLL) tumor cells [5].

Rowe et al. [6] classified the EBV-associated tumors into three types by expression of the EBV-encoded latent membrane protein (LMP) and nuclear antigen (EBNA). For example, in HD, LMP was expressed in the H & RS cells, while EBNA2 was absent [4]. In the B-lymphoblastoid cell lines, both were expressed, while in Burkitt's lymphoma both were absent [6].

Sequencing identified the viral BCRF-1 [viral (v) interleukin-10 (IL-10)] gene of EBV, a sequence that is highly homologous to the human IL-10 (hIL-10) gene [7]; it shares most functions with hIL-10, which was initially purified as a cytokine synthesis inhibitory factor

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TABLE I. EBV and Associated Antigens in Malignant Lymphoma

Diagnosis[a]	No. of cases	DNA analysis: Southern blot W	DNA analysis: Southern blot TR	DNA analysis: PCR	ISH[b] (EBER-1) ++	ISH[b] (EBER-1) +	ISH[b] (EBER-1) +/−	Immunological staining: LMP	Immunological staining: EBNA-2	Immunological staining: IL-10
B-cell lymphoma (total)	60	8	7	10	7	1	17	4	3	2[c]
Diffuse large (large/immunoblastic)	36	6	5	6	5	1	9	3	2	2[c]
Diffuse medium (small cleaved)	13	0	0	2	0	0	6	0	0	0
Follicular medium (small cleaved)	8	0	0	0	0	0	2	0	0	0
Burkitt (small noncleaved)	1	0	0	0	0	0	0	0	0	0
Anaplastic large	2	2	2	2	2	0	0	1	1	1
T-cell lymphoma (total)	46	5	3	14	0	5	22	6[d]	0	4[d]
ATLV(−) (subtotal)	29	4	2	11	0	4	11	5[d]	0	0
Diffuse large (large/immunoblastic)	5	0	0	0	0	0	2	0	0	0
Diffuse medium (small cleaved)	7	0	0	3	0	0	4	2[d]	0	2[d]
Diffuse pleomorphic (immunoblastic)	4	1	1	1	0	1	1	0	0	0
AILD-T	13	3	1[e]	7	0	3	4	3[d]	0	2[c]
ATLV(+) (subtotal)	17	1	1	3	0	1	11	1[d]	0	0
Diffuse large (large/immunoblastic)	5	1	1	1	0	1	4	1[d]	0	0
Diffuse medium (small cleaved)	7	0	0	1	0	0	5	0	0	0
Diffuse pleomorphic (immunoblastic)	5	0	0	1	0	0	3	0	0	0
Non-specific lymphadenitis	13	0	0	6	0	0	6	0	0	0

[a]Definitions in parentheses are according to the Working Formulation (WF); diffuse large (LSG) includes all diffuse large (WF) and some diffuse immunoblastic lymphoma (WF), which shows mild nuclear pleomorphism.
[b]++, > 50%; +, 2–50%; +/−, < 2%.
[c]One of two cases shows a few positive cells.
[d]A few cells are positive.
[e]Two bands.

[8,9]. In addition, vIL-10 has also been established as a new latency gene with a direct transformation-prerequisite function [10]. Study of the terminal repeat (TR) sequences of the EBV genome is considered to be useful as a means of assessing the clonality of the cellular population harboring the virus [11]. We thus investigated EBV infection in nodal non-Hodgkin's lymphoma using polymerase chain reaction (PCR), Southern blot analysis, and in situ hybridization. In addition, expression of the EBV-associated oncoproteins LMP, EBNA-2, and IL-10 were also analyzed.

MATERIALS AND METHODS

From this tissue specimens filed in the Department of Pathology, Fukuoka University, we selected lymph node specimens from 106 cases histologically diagnosed as non-Hodgkin's lymphoma. The lymphomas were divided into T- or B-cell type by both phenotype and genotype and then histologically subdivided by the lymphoma study group (LSG) classification. Almost all cases used have been previously reported [12]. The LSG classification is almost the same as the Working Formulation (WF) (Table I). We fixed the lymph nodes in either buffered formalin or B5 solution, embedded them in paraffin, and stained them with hematoxylin-eosin, Giemsa, and Gomori's silver impregnation. A portion of each lymph node was kept at −80°C for immunological analysis using monoclonal antibodies. We used 13 cases of lymph nodes with non-specific lymphadenitis (showing no history of infectious mononucleosis) as a control study.

Immunohistochemistry

Monoclonal antibodies for EBV-encoded LMP1 (Dakopatts, Copenhagen, Denmark) and EBNA2 (Novocastra, UK) were also employed. In addition, monoclonal antibodies for human and viral IL-10 (anti-h- and -vIL-10), which have a cross-reaction with both human and viral IL-10, and viral IL-10 (anti-vIL-10) (Pharmingen, San Diego, CA, USA) were also used. Antibodies to L26 (CD20), UCHL-1 (CD45RO) (Dako), LeuM1 (CD15) (Becton-Dickinson, Mountain View, CA, USA), and BerH2 (CD30) (Dakopatts) were also studied in the paraffin-embedded samples. A portion of each lymph node, kept at −80°C, was examined using monoclonal antibodies for T cells (CD1, CD2, CD3, CD4, and CD8), B cells (CD19 and CD20), and Ki1 (CD30) (Becton-Dickinson, Dakopatts, Pharmingen, or Novocastra).

In Situ Hybridization

EBV RNA in situ hybridization (ISH) was performed, with a 30-base oligonucleotide of the EBER-1 gene portion, using a previously described method [13]. In brief, 3 μm paraffin sections were deparaffinized in xylene,

dehydrated in ethanol, air dried, predigested with pronase, prehybridized, and hybridized overnight with a digoxigenin-labeled oligo-antisense probe (EBER-1), which was synthesized on the basis of a published report [13]. After washing, hybridization was detected with an antidigoxigenin antibody-alkaline phosphatase conjugate. We used a sense probe as a negative control.

Southern Blotting Analysis

Part of the frozen material was used for DNA isolation and gene analysis. Details of the examination methods have been reported previously [12]. The T-cell receptor (TCR) gene Cβ, Jγ, the immunoglobulin heavy chain (JH) gene, proviral DNA of human T-cell lymphotropic virus type 1 (HTLV-1) (full length; gag, pol, env, pX, LTR), the EBV gene (*Bam*HI W region; Enzo, Hudson, NY, USA), and EBV-TR repeat (kindly provided by Dr. K. Hirai, Department of Virology and Immunology, Tokyo Medical and Dental University, Tokyo, Japan) were used as probes. We digested DNA with restriction enzymes *Eco*RI, *Hind*III, or *Bam*HI.

Polymerase Chain Reaction

Isolated DNAs were used for PCR. We synthesized the specific primers to examine the presence of EBV on the basis of the published DNA sequence, which corresponded to the EBV W region [14]. After PCR amplification, one-tenth of the reaction mixture was then analyzed by Southern blot.

RESULTS

A total of 119 cases were examined: 106 with non-Hodgkin's malignant lymphoma (ML), and 13 with non-specific lymphadenitis. ML was categorized into T-cell lymphoma by the genotype of TCR and the phenotype of UCHL-1, CD2, CD3, CD4, and/or CD8 or B-cell lymphoma by JH, L26, CD19, and/or CD20. Anaplastic large cell lymphoma was identified by morphology and CD30 (Table I). HTLV-1 proviral DNA was found in 17 cases of T-ML.

EBV Presence: Correlation of Southern Blot, PCR, and ISH

EBV genomes and EBV-infected cells were found in 13 of 106 MLs (12%) by Southern blot analysis, in 24 of 106 MLs (23%) by PCR, and in 52 of 106 MLs (49%) by ISH (Fig. 1 and Table I). In non-specific lymphadenitis, EBV was found in 6 of 13 cases (46%) by PCR and ISH. The density of the DNA bands and amplified DNA and the number of ISH-positive cells varied, considered to be due to the varying numbers of EBV-infected cells. The sensitivity of ISH was the best, since the Southern blot and PCR targets consisted of only a few copies of the EBV-W region DNA in the EBV-infected cell, whereas the ISH target consisted of up to 10^7 copies of EBER-1 RNA [13].

EBV-Infected Cells: Distribution by ISH

EBV-infected cells could be found in 52 of 106 MLs (49%) by ISH. In non-specific lymphadenitis, EBV was found in 6 of 13 cases (46%) (Fig. 2 and Table I). Morphologically, the EBV-infected cells were confined to lymphoid cells. No other cell types (fibroblasts, endothelial cells, or histiocytes) showed any EBV infection.

Based on the number of EBV-infected cells, MLs were groups into three types: I) almost all lymphoma cells showed an EBV presence; II) some scattered lymphoma cells showed an EBV presence; and III) only a few cells showed EBV. Infected cells made up 50–100% of the entire lymphoma cell population in type I, 2–50% in type II, and under 2% in type III.

In 25 of 60 B-MLs, EBV-infected cells were found; 7 were type I, 1 was type II, and 17 were type III. In 27 of 46 T-MLs, EBV-infected cells were found; 5 cases were type II, and 22 were type III. In 6 of 13 lymph nodes with non-specific lymphadenitis, EBV-infected cells were found, and all were type III. Two of 60 B-MLs were metastases of pyothorax-associated lymphoma (PAL), and the two cases were type I.

EBV-Associated Products (LMP, EBNA-2, and vIL-10)

Expression of LMP was demonstrated on the cell membrane and cytoplasm (Fig. 3 and Table I). In addition, LMP was found in four B-MLs and six T-MLs. In four B-MLs, LMP was expressed in almost all EBER-1-positive lymphoma cells; however, LMP of T-MLs was found in some scattered lymphoma cells in T-MLs. The number of LMP-positive cells was less than the number of EBER-1-positive cells by ISH. Expression of vIL-10 was confined to the LMP-positive cases, and the number of vIL-10-positive cells was less than the number of LMP-positive cells. In the serial sections, vIL-10-positive cells were positive for LMP. Expression of EBNA-2 was found in only three B-MLs, and EBNA-2 was expressed in almost all the lymphoma cells. One of the three EBNA-2-positive cases was an aged patient with anaplastic large cell lymphoma; the other two had pyothorax-associated lymphoma (PAL).

Clonality of EBV-Infected Cells

Seven B-MLs and three T-cell lymphomas showed clonal TR bands. One case of T-cell lymphoma with angioimmunoblastic lymphadenopathy features (AILD-T) displayed two clonal bands, and the other showed a monoclonal band. The density of the bands varied, but it was related to the number of EBER-1-positive cells. In type II T-MLs, the band showed a very weak density,

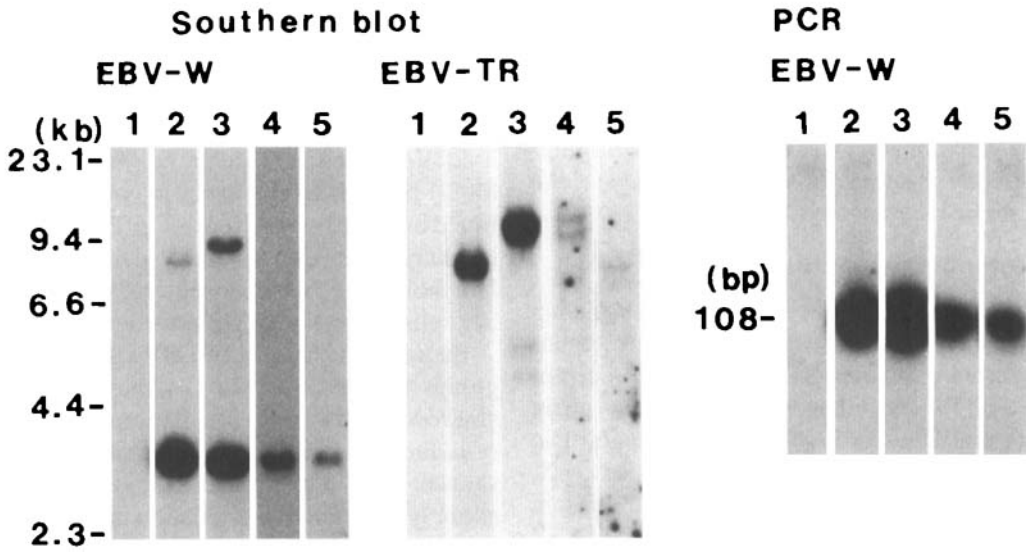

Fig. 1. A Southern bot and PCR analysis of the EBV genomes. In the Southern blot of EBV-W and TR, lane 1 is a negative control (placental DNA), lane 2 is a positive control (EBV+ lymphoepithelioma), lane 3 is B-ML (diffuse large), lane 4 is T-ML (AILD-T), and lane 5 is T-ML (ATLL). The *Bam*HI restriction enzyme is used. In the PCR analysis, lane 1 is a negative control, lane 2 is a positive control, lane 3 is B-ML, lane 4 is T-ML, and lane 5 is non-specific lymphadenitis.

and in type I B-MLs, the band showed a strong density (Fig. 1 and Table I).

DISCUSSION

The present study shows an unexpectedly high incidence of EBV infection in nodal non-Hodgkin's lymphoma (ML). By EBER-ISH, EBV infection was found in 52 of 106 cases of ML, but the number of the EBV-infected cells varied and the proportion of the infected cells ranged from 1% to 100%. From the number of EBV-infected cells, non-Hodgkin's lymphomas were grouped into three types: I) almost all lymphoma cells showed an EBV presence; II) some scattered lymphoma cells showed an EBV presence; and III) only a few cells showed EBV, probably due to latent EBV infection. In 25 of 60 B-MLs, EBV-infected cells were found; 7 were type I, 1 was type II, and 17 were type III. In 27 of 46 T-MLs, EBV-infected cells were found; no cases were type I, 5 were type II, and 22 were type III.

Previously 208 cases of B-MLs without human immunodeficiency virus (HIV) infection were investigated for an association with EBV infection in Europeans using PCR and ISH [15]. EBV was present overall in 26% (54/208). Through EBER-ISH, EBV could be localized in tumor cells of 27 cases (13%, 27/208), but the proportion of EBV-infected cells in the different cases varied between 1 and 100%. Morphologically, the 17 cases (8%) with over 80% EBV-infected tumor cells were either anaplastic large cell lymphomas, sporadic Burkitt's lymphomas, or high-grade lymphoma with plasmacellular differentiation. In these cases, EBV infection probably takes place before malignant transformation, whereas in the other cases, with a smaller number of infected cells, EBV infection may have occurred following malignant transformation [15]. Our Japanese nodal B-MLs displayed more frequent EBV infection than European cases, but the difference does not seem to be significant. Our seven cases (12%, 7/60) with over 50% EBV-infected tumor cells were also anaplastic large cell lymphomas or large cell lymphomas, which included PAL, or large cell lymphoma with plasmacellular differentiation.

EBNA-2 is a kind of oncoprotein that, in association with LMPs, plays an important role in B-lymphocyte transformation [16]. The cytotoxic T cells of the host recognize the LMP molecules and induce cell-mediated cytolysis. In the absence of LMP, the EBV-induced transformants cannot be lysed by cytotoxic T cells [15]. Rowe et al. [6] classified EBV-associated tumors into three types based on expression of LMP and EBNA. For example, in Hodgkin's disease, LMP was expressed, whereas EBNA-2 was absent (latency II) [4]. In the B-lymphoblastoid cell lines, both were expressed (latency III), and in Burkitt's lymphoma both were absent (latency I) [6]. In this study, LMP was found in four B-MLs and six T-MLs. In four B-MLs, LMP was expressed in almost all EBER-1-positive lymphoma cells, but LMP was found in only a few scattered lymphoma cells in T-MLs. Expression of EBNA-2 was found in only three B-MLs, in which EBNA-2 was expressed in almost all lymphoma cells. Three cases of EBNA-2-positive B-MLs, in which LMP was positive, were classified as latency III type, and the other seven LMP-positive B-MLs and T-MLs were latency II type. In addition, small EBV-infected bystander

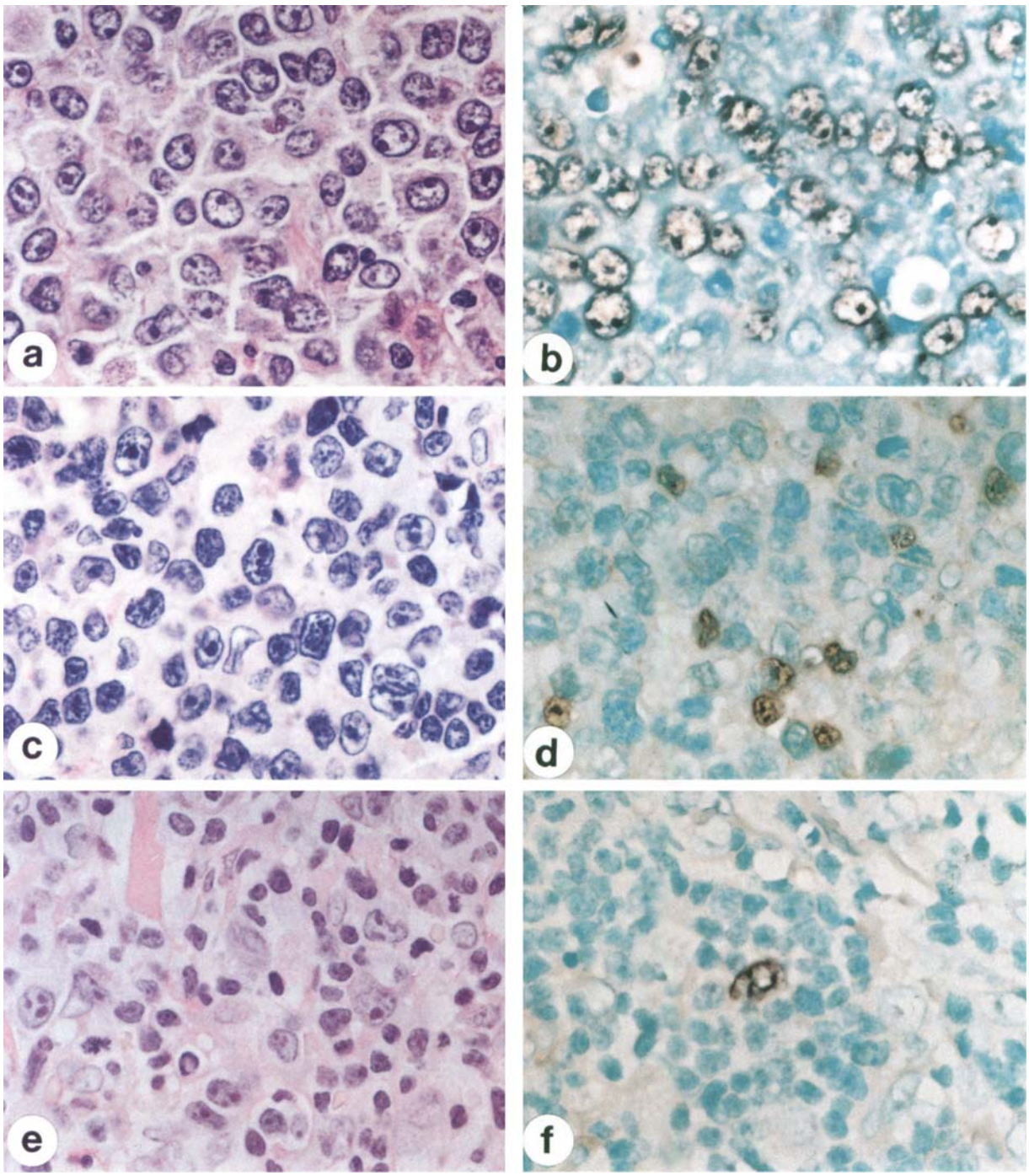

Fig. 2. Light microscopical appearance (a, c, e) and in situ hybridization (EBER-1) (b, d, f). a and b, c and d, and e and f are the same cases. A diffuse proliferation of atypical large lymphoid cells is found (a; B-ML), and almost all lymphoma cells show an EBV infection (b). A diffuse proliferation of lymphoma cells (c; ATLL) and some lymphoma cells show EBV (d). A diffuse proliferation of lymphoma cells (e; AILD-T); only one cell shows an EBV infection (f).

cells proved to be constantly LMP negative; they were thus probably latency I type. In our nodal lymphomas, no specific pattern of LMP and EBNA-2 expression was found.

PAL is a rare tumor associated with long-standing tuberculous pyothorax [17]. Most of these lymphomas are B-cell lymphomas and are closely associated with EBV. Both EBNA-2 and LMP were demonstrated in PAL, as in immune-deficient EBV-infected lymphoma [17]. Southern blot analysis showed a single terminal fragment

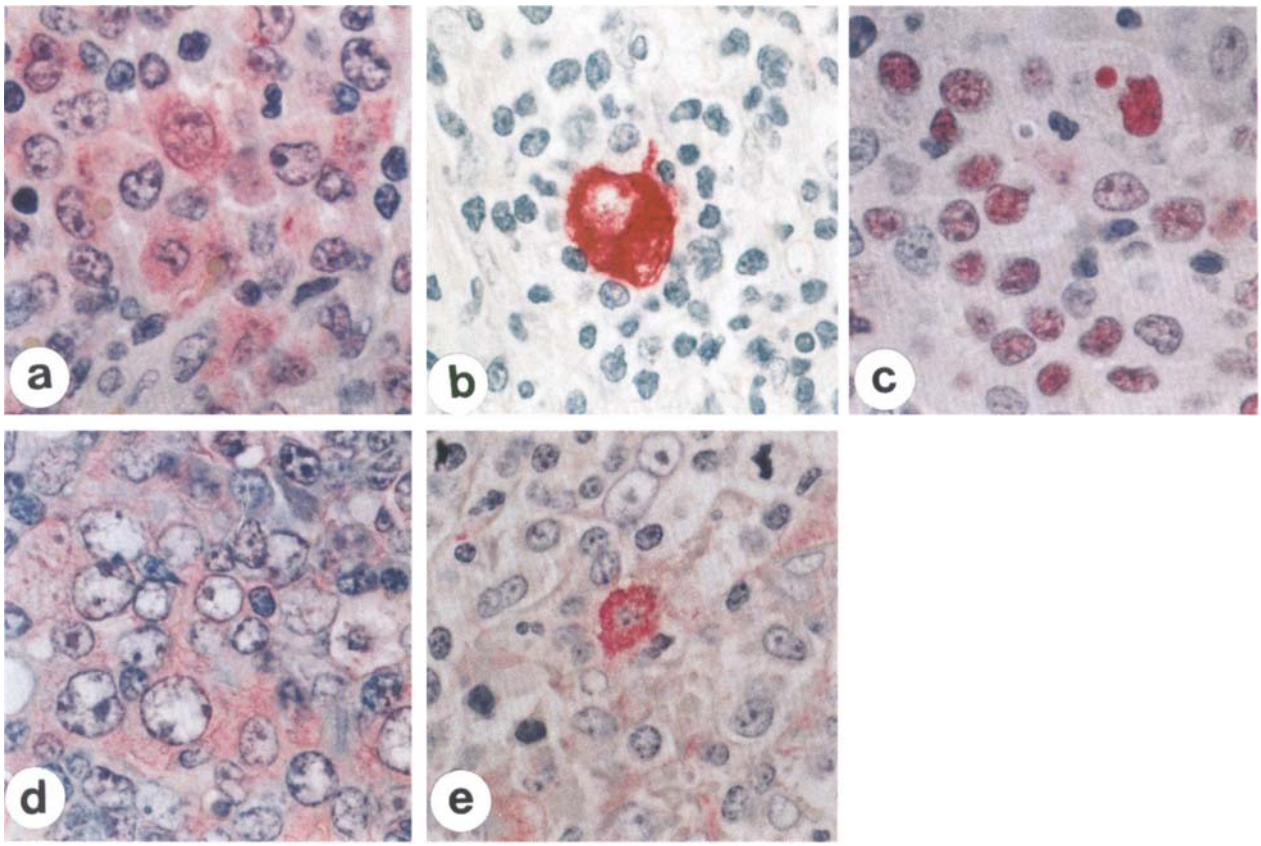

Fig. 3. Immunohistochemical staining. Staining for LMP shows a positive reaction in the cytoplasm of almost all lymphoma cells (a; B-ML) and a large immunoblast-like cell (b; AILD-T). EBNA2 is positive in the nuclei of almost all lymphoma cells (c; B-ML). IL-10 is positive inthe cytoplasm of almost all lymphoma cells (d; B-ML), and IL-10 is positive in a few cells (e; AILD-T).

in PAL. Our two cases also showed LMP, EBNA-2, and a clonal terminal fragment in each.

ATLL is a human malignancy associated with HTLV-I, which is a retrovirus [17]. There has also been a report in which the C3d receptor expression on the cell membrane of ATLL was detected [18,19]. Tokunaga et al. [5] reported that ISH indicated EBV in the nuclei of ATLL tumor cells in 16 of 96 cases (17%), while the number of EBV-positive cells varied. However, our ATLL study showed a more frequent EBV infection, which may be due to the sensitivity of the methods used.

Study of the TR sequences of the EBV genome is considered to be useful as a means of assessing the clonality of the cellular population harboring the virus [11]. For example, EBV-associated lymphoepithelioma of the nasopharynx represents a clonal expansion of a single EBV-infected progenitor cell [11]. TR analysis revealed the oligoclonal or monoclonal origin of B-cell lymphoma [20], polyclonal B-cell lymphoproliferation of infectious mononucleosis [21], and monoclonal T-cell lymphoproliferation in chronic active EBV infection [22]. In this study, all ten cases in which TR bands could be found showed clonal bands, but the density of the bands varied. The density related to the proportion of EBV-infected cells. In those cases in which TR was detected, LMP and EBNA-2 reaction were positive or negative and showed no specific patterns. The reason for this is uncertain; possibly clonal pathogenesis is not associated directly with either lymphomagenesis or the immune reactions of the hosts.

IL-10 was initially purified as a cytokine synthesis inhibitory factor, which inhibits many types of cytokine release including interferon-γ (IFN-γ), which is important in mediating cellular defenses against EBV infection [9]. IL-10 also exhibits strong DNA and amino acid sequence homology to BCRF1 (vIL-10), an open reading frame in the EBV genome [7]. vIL-10 has apparently conserved many functions of IL-10. The inhibitory activity would also likely benefit the virus, because both T cells and natural killer cells will permit an EBV infection and immortalization of the B cells [8]. Using a wide panel of EBV-positive and EBV-negative cell lines, it has been shown that EBV-positive B-cell lines derived from patients with the acquired immunodeficiency syndrome and

Burkitt's lymphoma secrete large quantities of IL-10. By contrast, EBV-negative B-cell lines do not express IL-10 [23]. LMP induced IL-10 production in EBV-carrying Burkitt's lymphoma lines, whereas EBNA showed no such induction [24]. In this study, the LMP-positive cases frequently showed expression of IL-10. Thus LMP probably induced IL-10 production in the EBV-infected cells. Expression of IL-10 did not differ between T-MLs or B-MLs.

Previously, 81 cases of T-ML occurring in HIV-negative Europeans were investigated to study EBV infection [25]. By EBER-ISH, the virus was located in the tumor cells of 30 cases, with the proportion of the infected cells ranging from 1% to 100%. The EBV infection ratio was different in the primary sites. Primary nasopharyngeal associated T-ML displayed frequent EBV infection (100%), whereas the rate in nodal T-ML was 59% and rarely demonstrated in cutaneous and enteropathy-associated T-ML [25]. Our Japanese nodal T-ML displayed frequent EBV infection (59%). The rate was same as in the previous European data. The pathogenesis of EBV in T-ML is thus probably not associated with any difference in race or geographic area.

The role of EBV in T-ML cannot be clearly understood unless the reason for the presence of EBV in only a proportion of and not all the neoplastic cells in most T-MLs studied is clarified. It is also necessary to explain the frequent coinfection of B cells in addition to EBV infection of the tumor cells [25]. EBER-ISH has shown that in other EBV-associated neoplasms, such as Burkitt's lymphoma and Hodgkin's disease, all tumor cells harbor the virus, which implies that such neoplasms are derived from a single lymphocyte with an EBV infection [25,26]. In our T-MLs, the monoclonal TR was found, but the density of the TR bands was weaker than those of the rearranged bands of the TCR gene. This means that the population of clonal EBV-infected cells was smaller than that of the tumor cells. In addition, EBV infected only a small portion of the tumor cell population. It appears to be more likely that EBV-positive T-ML cases are secondarily EBV-infected, i.e., after a malignant transformation. But there was also a possibility of EBV loss after malignant transformation [25]. Shimizu et al. [27] reported that, during cultivation of the EBV-positive Burkitt's lymphoma line Akata, EBV DNA is lost from some of the cells. The isolation of EBV-positive and EBV-negative clones with the same origin thus made it possible to examine the effects of EBV in Burkitt's lymphoma cells. It is thus possible that EBV infection is associated with the various stages of lymphomagenesis.

REFERENCES

1. Hanto DW, Gajl-Peczalska KJ, Frizzera G, Arthur DC, Balfour HH, McClain K, Simmons RL, Najarian JS: Epstein-Barr virus (EBV) induced polyclonal and monoclonal B cell lymphoproliferative disease occurring after renal transplantation. Clinical, pathologic, and virologic findings and implications for therapy. Ann Surg 198:356, 1983.
2. Hamilton-Dutoit SJ, Pallesen G, Franzmann MB, Karkow J, Black F, Skinhoj P, Pedersen C: AIDS-related lymphomas. Histopathology, immunophenotype and association with Epstein-Barr virus as demonstrated by in situ nucleic acid hybridization. Am J Pathol 138:149, 1991.
3. Weiss LM, Movahed LA, Warnke RA, Sklar J: Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. N Engl J Med 32:502, 1989.
4. Herbst H, Dallenbach F, Hummel M, Niedobitek G, Pileri S, Müller-Lantzsch N, Stein H: Epstein-Barr virus latent membrane protein expression in Hodgkin and Reed-Sternberg cells. Proc Natl Acad USA 88:4766, 1991.
5. Tokunaga M, Imai S, Uemura Y, Tokudome T, Osato T, Sato E: Epstein-Barr virus in adult T cell leukemia/lymphoma. Am J Pathol 143:1263, 1993.
6. Rowe M, Lear AL, Croom-Carter D, Davies AH, Rickinson AB: Three pathways of Epstein-Barr virus gene activation from ENBA1 positive latency in B lymphocytes. J Virol 66:122, 1992.
7. Vieira P, DeWaal-Malefty R, Dang MN, Johnson KE, Kastel-Stein R, Fiorentino DF, De Vries JE, Roncarolo MG, Monsmann TR, Moore KW: Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: Homology to the Epstein-Barr virus open reading frame BCRF-1. Proc Natl Acad Sci USA 88:1172, 1991.
8. Zlotnik A, Moore KW: Interleukin 10. Cytokine 3:366, 1991.
9. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE: Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes. An autoregulatory role of IL-10 produced by monocytes. J Exp Med 174:1209, 1991.
10. Miyazaki I, Cheung RK, Dosch HM: Viral interleukin 10 is critical for the induction of B cell growth transformation by Epstein-Barr virus. J Exp Med 178:439, 1993.
11. Raab-Traub N, Flynn K: The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. Cell 47:883, 1986.
12. Ohshima K, Kikuchi M, Eguchi F, Masuda Y, Sumiyoshi Y, Mohtai H, Takeshita M, Kimura N: Analysis of Epstein-Barr viral genomes in lymphoid malignancy using Southern blotting, polymerase chain reaction and in situ hybridization. Virchows Arch [B] 59:383, 1990.
13. Chang KL, Chen YY, Shitata D, Weiss LM: Description of an in situ hybridization methodology for detection of Epstein-Barr virus RNA in paraffin-embedded tissues, with a survey of normal and neoplastic tissues. Diagn Mol Pathol 1:246, 1992.
14. Saito I, Servenius B, Compton T, Fox RI: Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjögren's syndrome. J Exp Med 169:2191, 1989.
15. Hummel M, Anagnostopoulos I, Korbjuhn P, Stein H: Epstein-Barr virus in B-cell non-Hodgkin's lymphomas: Unexpected infection patterns and different infection incidence in low- and high-grade types. J Pathol 175:262, 1995.
16. Wang F, Gregory CD, Sample C, Rowe M, Liebowitz D, Murray R, Rickinson AB, Kleff E: Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. J Virol 64:2309, 1990.
17. Sasjima Y, Yamabe H, Kobashi Y, Hirai K, Mori S: High expression of the Epstein-Barr virus latent protein EB nuclear antigen-2 on pyothorax-associated lymphomas. Am J Pathol 143:1280, 1993.
18. Uchiyama T, Yodi J, Sagawa K, Takatsuki K, Uchino H: Adult T-cell leukemia: Clinical and hematologic features of 16 cases. Blood 50:481, 1977.
19. Kai C, Okada N, Okada H: Expression of CR2 (C3d receptor) on the cell membranes of adult T cell leukemia. Jpn J Cancer Res 79:805, 1988.
20. Cleary ML, Nalesnik MA, Shearer WT, Sklar J: Clonal analysis of transplant-associated lymphoproliferations based on the structures of genomic termini of the Epstein-Barp virus. Blood 72:349, 1988.

21. Brown MA, Liu C, Garcia CR, Wang YF, Griffith A, Sparkes RS, Calame KL: Clonal origins of lymphoproliferative disease induced by Epstein-Barr virus. J Virol 58:975, 1986.
22. Ishihara S, Tawa A, Yumura-Yagi K, Murata M, Hara J, Yabuchi H, Hirai K, Kawa-Ha K: Clonal T-cell lymphoproliferation containing Epstein-Barr (EB) virus DNA in a patient with chronic active EB virus infection. Jpn J Cancer Res 80:99, 1989.
23. Benjamin D, Park CD, Sharma V: Human b cell interleukin 10. Leuk Lymphoma 12:205, 1994.
24. Nakagomi H, Dolcetti R, Bejarano MT, Pisa P, Kiessling R, Masucci MG: The Epstein-Barr virus latent membrane protein-1 (LMP-1) induces interleukin-10 production in Burkitt lymphoma lines. Int J Cancer 57:240, 1994.
25. Korbjuhn P, Anagnostopoulos I, Hummel M, Tiemann M, Dallenbach F, Parwaresch MR, Stein H: Frequent latent Epstein-Barr virus infection of neoplastic T cells and bystander B cells in human immunodeficiency virus-negative European peripheral pleomorphic T-cell lymphomas. Blood 82:217, 1993.
26. Herbst H, Steinbrecher E, Niedobtitek G, Finn T, Young LS, Brooks L, Müller-Lantzsch N, Stein H: Distribution and phenotype of Epstein-Barr virus infected cells in Hodgkin's disease. Blood 80:484, 1992.
27. Shimizu N, Tanabe-Tochikura A, Kuroiwa Y, Takada K: Isolation of Epstein-Barr virus (EBV)-negative cell clones from the EBV-positive Burkitt's lymphoma (BL) line Akata: Malignant phenotypes of BL cells are dependent on EBV. J Virol 68:6069, 1994.